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Toxicological Effects of Chlorite in the Mouse

by Gary S. Moore* and Edward J. Calabrese*

When exposed to a maximum level of 100 ppm chlorine dioxide in their drinking water, neither A/J or C57L/J mice exhibited any hematologic changes. Chlorite exposure under similar conditions produced increases for red blood cells in osmotic fragility, mean corpuscular volume, and glucose-6-phosphate dehydrogenase activity for both strains. Chlorite exposure of pregnant A/J mice resulted in a significant decrease in the weight of pups at weaning and a lower average birth to weaning growth rate. Mice exposed to as much as 100 ppm sodium chlorite ($NaClO_2$) in their drinking water for up to 120 days failed to demonstrate any histopathological changes in kidney structure.

Introduction

The primary method of water disinfection in the United States employs chlorine. Studies, however, have shown that chlorine interacts with organics in the water to form trihalomethanes (*1,2*). One of the trihalomethanes is chloroform, which has been found to be carcinogenic in rats (*3*). It has been postulated that chlorinated water supplies may increase the risk of cancer to humans (*4–7*); such compounds are formed to a lesser extent in water disinfected with chlorine dioxide (*8*). While toxicological studies have revealed limited physiologic effects from chlorine dioxide, concern has now been directed towards the end products of such disinfection which include chlorate and chlorite (*7,8*). Chlorites appear in concentrations of up to 50% of the chlorine dioxide demand where chlorine dioxide is used for disinfection (*8*). Blood destruction, nephritis, and methemoglobinemia have developed in humans after acute poisoning with large doses of chlorate (*9,10*). It was suggested that in chronic poisoning from chlorate, uremia, anemia, and nephritis might develop. Chlorite is thought to be a more potent oxidant stressor to blood than chlorate (*11*). Heffernan (*12,13*) reported that chlorite belonged to a class of oxidant compounds that stimulate the reduced glutathione (GSH) levels and the development of hemolytic anemia in animal models.

In attempting to establish minimal health effect levels of oxidants (chlorine dioxide and chlorite) on erythrocytes, it is important to consider the effects of such stressors on potential high risk groups. A large group that falls into this category are persons with lowered G6PD (glucose-6-phosphate dehydrogenase) activity. G6PD-deficient cells have a reduced ability to produce NADPH via the pentose phosphate pathway (PPP), and consequently less GSH is formed. Since GSH is the primary mechanism of defense of the red blood cell against oxidant stress, then persons with deficient G6PD levels have a lowered capacity for protection against oxidants (*14, 15*) and virtually 100 million males throughout the world belong in this category (*14*). Among the studies reported here are those designed to evaluate the effects of chlorine dioxide and chlorite on erythrocytes of mouse strains with differential G6PD activity (i.e., the A/J strain has three times the activity of the C57L/J strain).

Another potential high risk group would likely include newborns. The potential effects of chlorite on human newborns may be exaggerated because: (1) infants consume nearly three times as much liquid per unit of body weight than adults (*16*); (2) infants are born with hemoglobin F (fetal hemoglobin), a form that is readily oxidizable to methemoglobin (*17*); (3) infants have a lower capacity than adults to reduce methemoglobin enzymatically to

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hemoglobin (*18*); (4) newborn infants are characteristically low in vitamin E (*18*), an important antioxidant compound, because there is little placental transfer of vitamin E.

There is also evidence that the fetus may be at increased risk to oxidant stressors. Shuval and Gruener (*19*) demonstrated that nitrites can be transferred to the fetus *in utero* causing an elevation of MetHb in fetal blood. When administered in drinking water to pregnant rats, 2000–3000 mg/l. of $NaNO_2$ resulted in a decreased litter size, increased mortality of pups in the first three weeks and a lagging of growth rate despite having equal birthweight to controls.

Sodium chlorate has been shown to produce methemoglobin *in vitro* and *in vivo* when administered orally (*9,10,20*). Oral acute doses of sodium chlorate have resulted in hemolysis, nephritis, methemoglobinemia, and death in man and various species of test animals (*9*). Since previous studies have shown chlorate to cause renal damage, it was hypothesized that chlorite, because of its more potent oxidant capabilities, may also be potentially harmful to the kidney.

In order to test the above hypotheses, a number of experiments were performed on laboratory animals. The experiments were designed to measure the effects of orally administered chlorite on blood characteristics, kidney structure, as well as the fertility, and the growth rate, mortality and development of newborns.

Methods

Animal Care

Two strains of mice, with "normal" (A/J) and low (C57L/J) levels of G6PD activity, were obtained from the Jackson Laboratory, Bar Harbor, Maine. The mice were individually housed in stainless steel cages equipped with constant feed food hoppers and 50 ml calibrated watering devices. The cages were kept under controlled conditions of 12 cycles of light and dark (9 A.M. - 9 P.M. light, 9 P.M. - 9 A.M. dark), and a temperature of $74 \pm 2°F$. Animals were held at these conditions for a one-week period after receipt in order to assess their health and allow for acclimation. Animals were randomly allocated to each of the treatment categories, according to a random scheduling of treatments. Treatment periods of 12 hr occurred during the dark cycle in order to minimize decomposition of ClO_2 by light and to coincide with the nocturnal activity period of the mice.

The watering devices were filled with the appropriate treatment solutions and a reading taken of the volume both before and after the treatment period. The bottles were removed at the end of this period, and no other water provided until the next treatment period. Average daily consumption of liquid was computed for each mouse, and an overall average computed for each treatment category.

Mice were fed *ad libitum* a balanced rodent diet (Purina Rodent Chow) composed of not less than 23% crude protein, 4.5% crude fat, 6.0% crude fiber, 8.0% ash, and 2.5% minerals.

Experimental Procedures

Complete Blood Count (CBC). The Royco Cellcrit IV Hematology System, consisting of Model 920–A cell counter, Model 720–A hemoglobinometer and Model 365–A diluter, was used to determine red blood cell count (RBC), hematocrit (HCT), white blood count (WBC) and hemoglobin concentration (HBG). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were then computed from these data.

Quality assurance of equipment performance was checked on a daily basis by means of commercial standards, and equipment performance was measured by comparison of automated and manual hematocrits of the same blood sample.

Reticulocyte Count. Staining of reticulocytes was accomplished according to the procedure outlined by Seiverd (*21*). A 1% physiological saline solution of Brilliant Cresyl Blue was mixed with an equal quantity of whole blood and allowed to stand for 20–30 min. The contents of the tube were then remixed and a smear made. The smear was observed under oil immersion and a total of 500 cells counted. The reticulocytes were expressed as a percentage of the total number of cells counted.

Glucose-6-Phosphate Dehydrogenase Assay (G6PD). G6PD activity was determined by a colorimetric procedure using Calibiochem (10933 N. Torrey Pines Rd. LaJolla, Calif. 92037) G6PD reagents and a Perkin-Elmer Coleman 55 spectrophotometer equipped with a flow-through temperature-controlled cell, a Coleman 5–100 enzyme calculator, and a Coleman 47 B.C.D. printer. The G6PD activity was calculated in terms of International Units (I.U.) per gram of hemoglobin in whole blood hemolysate.

Osmotic Fragility (Automated). Osmotic fragility was determined using the American Optical Fragilograph (Clinical Instruments Co., Dudley, MA 01570). A 10 µl portion of whole blood was diluted in 7.5 ml of 0.68% isotonic buffered saline (Isoton II diluted to obtain 0.68%), yielding a 1:750 dilution. The instrument was calibrated with 1.5 ml of the prepared cell suspension, added to a cuvette, and the osmotic fragility determined.

Glutathione (GSH) Assay. Reduced glutathione was determined by using Glutathione and Erythrozyme Assay (from Princeton Biomedix Inc., Princeton, NJ) using a reagent solution containing 5,5-dithiobis(2-nitrobenzoic acid) (DTNB) which reacts with glutathione to form a highly colored yellow ion.

Statistical Analysis

Analysis was performed by using the BMDP2V and SPSS ONEWAY (*22*) programs. The two-way ANOVA table was produced by the BMDP program with the three previously mentioned hypotheses either accepted or rejected, based upon the p values of the F test ($p = 0.05$). If a hypothesis was rejected, contrasts were constructed and with separated variances computed with accompanying p values. These contrasts were run on the SPSS ONEWAY program (*22*). The Bartletts and Cochrans tests for equal variances were also computed by using the SPSS ONEWAY program.

Experimental Design

Effect of Chlorite on Chlorine Dioxide on Blood Characteristics. Twenty A/J and 20 C57L/J mice were each divided into two groups of 10 mice with each strain being exposed to 0.0 ppm (10 mice) and 100 ppm (10 mice) chlorine dioxide in their drinking water for 30 days. The animals were lightly etherized and then decapitated by means of a guillotine. The blood was collected into 100 μl of 0.6% sodium heparin (Sigma) prior to analysis.

Both A/J ($N = 63$) and C57L/J ($N = 59$) mice were each divided into four groups and exposed to sodium chlorite at 0.0, 1.0, 10.0 and 100.0 ppm in their drinking water for 30 days (Table 1). At the end of the exposure period, blood was collected and analyzed as previously indicated. In addition to measuring the effects of several blood parameters including GSH levels, the weight change over 30 days and the average volume of water consumed per day was determined for each animal.

Effect on Conception Rate and Litters of A/J Strain Mice. Ten- to 12–week-old female A/J mice were grouped by ten in an 11.5 in. long × 7.5 in. wide × 5 in. deep polycarbonate breeding cage for one week in order to synchronize ovulation. Females were then randomly mated one to one with A/J males of a similar age and checked for vaginal plugs on the mornings of the next four days. Males were removed from females at the appearance of plugs. Date of plugging, as well as weight of sire and dam were recorded. Females that were not plugged were grouped for a week before breeding again.

Plugging was considered to be day one of pregnancy and females were placed in fresh breeding cages with wood chips for the duration of their pregnancy and lactation. At plugging, females were randomly assigned to either a control group which was given distilled water or a treatment group which was given 100 ppm sodium chlorite (Matheson, Coleman and Bell, Norwood, OH 45212) in distilled water. A dose of 100 ppm ClO_2^- was selected, since other studies (*12,13,23*) have shown that effects on hematological parameters are first observed at concentrations of chlorite reaching 50 to 100 ppm. Females remained on the specified water during gestation and lactation periods, and water intake was monitored during this time.

At parturition each litter was examined and data collected. Individual birth weights were taken and pups were weighed once a week until weaning at 28 days. Statistical analysis was performed on the data collected for each litter as well as water consumption and rate of positive pregnancy for each group.

Effects of Renal Structure. Water used for all treatment and control groups was processed by a Continental Water Deionization System (Millipore Corp., Bedford, Mass) and buffered with 1/30 M Sorenson's phosphate buffer (*24*) to maintain a pH of 7.17. Treatment water was prepared by the addition of appropriate amounts of $NaClO_2$ (Matheson Coleman and Bell, Norwood, Ohio) to buffered "Continental" water. Water used for control groups was prepared in a similar fashion with the addition of NaCl (Sigma Chemical Co., St. Louis, MO) instead of $NaClO_2$. All water for treatment and control mice was available *ad libitum*, and the amount of water consumed was recorded at every fresh change. Measurement of chlorite ion concentration in freshly prepared treatment water was performed using the DPD ferrous titrimetric method of Palin (*25*).

All mice were weighed and randomly allocated to specific time-exposure and treatment groups. Lengths of exposures of treatment and control mice were divided into three time-exposure periods: one group of 55 mice for 30 exposure days, one group of 55 mice for 90 exposure days, and one group of 60 mice for 180 exposure days. The five treatment groups within each time-exposure group were: 0.0 ppm $NaClO_2$ as a negative control, 4.0 ppm $NaClO_2$ (2.98 ppm as ClO_2^-), 20.0 ppm $NaClO_2$ (14.92 ppm as ClO_2^-), 100.0 ppm $NaClO_2$ (74.59 ppm as ClO_2^-), and 100.0 ppm sodium chloride (NaCl) as a high salt control.

Upon completion of their respective treatment periods, all animals were weighed and killed by cervical dislocation. Both kidneys were excised, weighed, divided longitudinally into two hemisections, and placed in appropriate fixatives for subsequent light microscopy and/or transmission electron micros-

copy. In preparation for light microscopy, portions of renal tissue were fixed for 12 hr in Helly's solution and subsequently chromated for 12 hr in a 3% potassium dichromate solution (*24*). Kidney hemisections were embedded in paraffin, cut in 5 μm sections, and stained with hematoxylin and eosin-phloxine B. Renal tissue from three randomized mice in each group were processed for electron microscopy. Portions of renal tissue were cut in 1.5 cm blocks and fixed overnight at 4°C in 2.6% glutaraldehyde in Millonig's phosphate buffer at pH 7.2 (*26*). The renal tissue was then finely diced and post-fixed for 1 hr in Millonig-buffered 1% osmium tetroxide solution. After Epon embedding and ultrathin sectioning, the tissues were stained with 7% uranyl acetate and lead citrate (*27*). Electron microscopy was performed by use of a Joel 100–S electron microscope at 80 kV.

Results

Effect of Chlorine Dioxide and Chlorite on Blood Characteristics

A total of 11 hematologic parameters were measured for effects of chlorine dioxide exposure. Mice exposed to sodium chlorite for 30 days exhibited treatment effects for three of the eleven hematologic parameters (Table 1). Specifically, there was an increase in G6PD activity, MCV (mean corpuscular volume) and osmotic fragility for both strains of mice exposed to the 100.0 ppm chlorite, whereas no significant differences from controls occurred for the 1.0 and 10.0 ppm chlorite exposures.

It is also evident from analysis of variance (Table 1) that there is a significant difference between strains for both G6PD activity ($p < 0.001$) and osmotic fragility ($p < 0.001$). There were no significant strain versus treatment interactions, indicating that both strains acted similarly to chlorite exposure with respect to the parameters measured. These differences in hematological parameters from treatment could not be explained by weight loss or gain ($p = 0.146$), or by differences in water consumption ($p = 0.291$) since there were no significant treatment effects with respect to these parameters.

Effect of Chlorite on Conception Rate and Litters of A/J Strain Mice

The means, standard deviations and *t*–results are presented for control and treatment groups in Table 2. The average number of pups born alive in each litter was 5.4 for controls and 5.0 for the treatment group ($p = 0.33$). The average number of pups alive at weaning (4 weeks after birth) was greater at 4.2 pups for controls than for the 3.4 pups in the treatment group ($p = 0.24$). In contrast to the number of living pups, the average number of pups per litter dead at birth was 1.5 for controls and 2.0 for treatment animals ($p = 0.25$). The average number of pups per litter that died after birth but before weaning was 1.3 for controls and 1.9 for the treatment group ($p = 0.214$).

The control litters had an average live birth litter weight of 1.27 g compared with 1.17 g for the treatment group ($p = 0.13$). Two variables where statistical significance was reached includes the averaged weight of pups at weaning and the average birth to weaning growth rate. The birth to weaning growth rate was 0.336 g/day for treatment animals while being significantly greater at 0.408 g/day for

Table 1. Analysis of variance and cell means for selected hematologic variables of A/J and C57L/J mice exposed to 0.0, 10.0, and 100.0 ppm of sodium chlorite.

		Cell means for			Analysis of variance results[a]			
Parameter	Treatment, ppm NaCl	A/J strain	Na	C57L/J strain	*N*	S	T	ST
G6PD activity, IU/g of hemoglobin in whole blood	0.0	6.10	16	1.88	14			
	1.0	6.05	12	1.74	11	0.000	0.000	0.902
	10.0	5.90	12	1.82	11			
	100.0	6.50	23	2.13	23			
Mean corpuscular volume	0.0	48.91	16	49.89	14			
	1.0	48.70	12	49.47	11	0.100	0.008	0.844
	10.0	48.58	12	49.25	11			
	100.0	50.39	23	50.55	23			
Osmotic fragility	0.0	0.440	12	0.458	11			
	1.0	0.440	12	0.453	11	0.000	0.084	0.875
	10.0	0.438	12	0.458	11			
	100.0	0.452	12	0.465	11			

[a] *N* = number of mice tested per cell. Probability *F* exceeded for strain differences (S); treatment (T) differences; and strain versus treatment (ST) interactions. When retested by orthogonal contrasting, significance was achieved ($p = 0.05$) for individual treatments.

Table 2. Differences between control (0.00 ppm chlorite) and treatment (100 ppm chlorite) groups for ten variables.[a]

Variable	Control group			Treatment group			t-test[b]		
	Mean	S	N	Mean	S	N	t	df	p
Liver litter size	5.4	2.5	21	5.0	3.3	12	0.42	31	0.339
No. alive at weaning	4.2	2.4	19	3.4	3.7	12	0.73	29	0.237
Avg. pup weaning weight	12.5	1.6	16	10.7	2.4	7	2.06	21	0.026
Birth-weaning growth rate	0.408	0.055	16	0.336	0.082	7	2.11	21	0.024
Gestation time	20.2	0.68	9	19.71	1.30	12	1.16[b]	17.1[b]	0.110
Breeding weight of mother	21.84	2.16	13	21.03	1.57	12	1.07	23	0.149
Age of mother at parturition (weeks)	18.0	3.9	21	18.5	4.21	12	–	–	–
Average liver birth litter weight	1.27	0.380	20	1.17	0.113	10	1.15[b]	24.7[b]	0.130
No. dead at birth	1.50	2.01	20	2.00	2.04	12	–0.68	30	0.252
No. survivors dying before weaning	1.32	1.77	19	1.90	2.02	10	–0.81	27	0.214

[a] S = standard deviation; N = number of litters.

[b] t-separate statistic, with adjusted degrees of freedom, are presented, since the variances of the control and treatment groups are shown to be unequal by the F-test ($p = 0.10$). Results are based on a one-sided test (H_A: mean for controls litters > mean for treatment litters).

Table 3. Analysis of variance for body weight (BW) change, kidney weight (KW), kidney: body weight ratio and weekly water consumption.

Parameter measured	No. of mice	Analysis of variance[a]		
		L	T	$L \times T$
Body weight change	167	0.000	0.690	0.808
Kidney weight	167	0.000	0.602	0.331
KW:BW ratio	167	0.103	0.348	0.602
H_2O consumption	166	0.023	0.948	0.519

[a] Probability F exceeded for length of exposure differences (L), treatment differences (T), and length of exposure versus treatment interactions ($L \times T$).

the control group ($p = 0.024$). Further, the average weight at weaning was 10.7 g for the treatment group and 12.5 g for the control group ($p = 0.026$).

The conception rate was also determined for control and treatment groups. All females positive for vaginal plugs were randomly placed into control and treatment groups. Not all plugged females conceived or produced litters. The percentage of dams that were plugged that also produced litters was defined as the conception rate. The conception rate for the treatment group was 39% and for controls was 56%.

Since differences between groups in the weight of dams, age, gestation time, or the amount of water consumed might have influenced the development of pups, these variables were measured. The gestation time of dams was not statistically different between control and treatment groups ($p = 0.110$). The average breeding weight of the treatment and control dams was 21.03 and 21.84 g, respectively ($p = 0.149$), while the age of mothers at parturition was 18 weeks for controls and 18.5 weeks for the treatment group. Finally, the level of water intake between control and treatment groups was measured. The water consumed during lactation is usually greater than that consumed during gestation, so these figures are presented separately. The control dams consumed an average of 5.9 ml/day during gestation and 15.6 ml/day during lactation. The treatment group consumed 6.2 ml/day of chlorite-treated water during gestation and 15.3 ml/day during lactation. The difference between treatment and control groups in the amount of water consumed was not significant for gestation period ($p = 0.08$) or lactation period ($p = 0.87$).

Effect of Chlorite Exposure on Kidney Structure

Two-way analysis of variance revealed no statistically significant treatment-related differences for any of the four physical parameters measured (body weight change, kidney weight, percent kidney to body weight ratio and weekly water consumption). Statistically significant length of exposure differences were observed in body weight change, kidney weight, and weekly water consumption; but a significant difference was absent in the percent kidney to body weight ratio (Table 3). Additionally, no significant interaction occurred between length of treatment exposures and treatment levels of any of the four parameters.

Chi-square analysis of mortality rates indicated that these rates were not significantly different between the 30-, 90- and 180-day groups nor between the five various treatment levels.

Macroscopically, the kidneys were normal. The kidneys' outer surfaces were smooth, encapsulated, and pinkish-brown in color. No external lesions or growths were observed. Internally, the kidneys were unilobar with a dark red medulla and a pinkish-red cortex. No internal lesions, growths or obstructions were observed.

Microscopic examination using light and transmission electron microscopes revealed no evidence of glomerular, vascular, obstructive or tubular disease. Glomeruli, vasculature, and tubular networks were free from significant nephritis, nephrosis, vascular sclerosis, and obstructions. Ultrastructural or minimal changes were not found.

Discussion

Based upon the data in this study and a previous one (*23*), it appears that exposure of A/J and C57L/J mice to 100 ppm of chlorite for 30 days produces increases in G6PD activity, mean corpuscular volume, osmotic fragility and acanthocytosis (regular deformations or projections on the cell surface). *In vitro* studies by Heffernan (*11,12*) revealed that incubation of human and rat erythrocytes with increasing concentrations of ClO_2^- produced a progression of alterations in the erythrocyte morphology. Concentrations of 7.4×10^{-4} *M* to 7.4×10^{-3} *M* ClO_2^- produced multiple sharp pointed deformations on the membrane similar to effects seen in our *in vivo* studies. *In vivo* studies by Abdel-Rahman et al. (*28*) showed morphologic changes in the erythrocytes of rats and chickens drinking ClO_2, ClO_2^-, and ClO_3^- daily for 3 months. These changes were in the form of crenated spheres (echinocytes) with distortions in the middle of the erythrocytes at 100 mg/l. of ClO_2^-. The morphologic changes to erythrocytes produced by *in vivo* or *in vitro* ClO_2^- exposure appear consistent among the results of different experimenters with the *in vivo* threshold at 100 mg/l. of ClO_2^-.

The studies of both Heffernan et al. (*13*) and Abdel-Rahman et al. (*28*) demonstrated a decrease in GSH levels of erythrocytes after *in vivo* exposure of rats to chlorite. Neither the A/J or C57L mice in our study revealed a similar decrease in GSH. However, G6PD activities were elevated, indicating that in the mice adaptation may have been sufficient to prevent measurable decreases in GSH. This did not preclude damage to cell membranes which may be protected by other mechanisms.

Heffernan et al. (*13*) reported that when rats were fed sodium chlorite, the levels of GSH were the most markedly affected of the blood parameters and declined in dose-response relation to chlorite exposure, with levels of chlorite at 60 ppm causing decreases of approximately 20% in GSH levels. Additionally, decreases in hemoglobin, red cell count and packed cell volume were also evident in the study of Heffernan et al. (*13*), indicating a mild hemolytic anemia. The present studies on mice (*23*) exposed to 100 pm chlorite indicated an increase in MCV (mean corpuscular volume), osmotic fragility, G6PD activity and the number of acanthocytes. Recent *in vitro* studies by Moore et al. (*29*) have revealed that human G6PD-deficient red cells are more susceptible to chlorite-induced decreases in GSH than are cells of humans with normal G6PD activity. Future studies are needed to quantify to what extent G6PD deficients may be at risk to chlorite induced RBC alteration in normal exposure conditions.

It should be emphasized that while the activity of A/J strain was three times that G6PD activity of the C57L/J strain, a value comparable to the ratio of normal to deficient human RBC G6PD activity, the absolute comparisons are quite dissimilar. That is, the C57L/J mice (i.e., the so-called "deficient" strain) have RBC G6PD activity similar to that of normal humans even though they showed only 1/3 the activity level of the A/J strain. Thus, the C57L/J strain did not represent a true deficient in absolute terms. In fact, their ability to increase GSH levels following chlorite stress suggests the ability to adapt to oxidant stress. Likewise, unpublished investigations in our laboratory have shown that the C57L/J mice were markedly resistant to primaquine, an oxidant drug which is known to induce hemolytic anemia in G6PD deficient humans (*15*). It thus seems that the hypothesis that G6PD deficiency enhances the occurrence of chlorite-induced hemolysis in humans remains to be evaluated via a model with a deficiency comparable in absolute terms to the human deficient condition and/or epidemiologically.

Our studies of chlorite exposure on conception rate and litters of A/J mice have demonstrated that there are no effects of ingested chlorite ion (100 ppm) on pregnant dams when measuring gestation time, breeding weight or age at parturition. Consequently, these variables do not appear to confound the results. Additionally, there is no significant difference in litter size, which also rules this out as a confounding factor in influencing weaning weight and birth-weaning growth rate. Although the number of pups alive at weaning was somewhat greater for the controls, this difference again was not significant. It would be expected that fewer mice in a litter either at birth or prior to the weaning process would result in greater weight gains. How-

ever, there were fewer in the treatment groups alive at weaning, yet they weighed significantly less on the average than controls, and had less weight gain. These results appear similar to those of Shuval and Gruener (*19*) who reported that pups of dams while drinking water with nitrite (1000–3000 mg/l.) experienced increased mortality in the first three weeks and lagged behind in growth, despite having equal birth weights to controls. The conception rate of the dams drinking chlorite-treated water was reduced 17% compared to controls. These findings indicate that chlorite ion at 100 ppm concentration is capable of reducing the conception rate of A/J mice and of retarding the growth rate of A/J pups through weaning. Levels of 100 ppm chlorite represent a 10- to 100-fold increase over expected concentrations in drinking water and additional studies are being undertaken to determine minimal health effect levels. The series of sodium chlorite doses administered orally in drinking water did not result in any detectable toxic damage to the kidneys of C57L/J male mice over the 60-, 90- or 180-day exposure periods.

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